THE EXPERIMENTAL PRODUCTION OF CORONARY INSUFFICIENCY IN DOGS

by

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INTRODUCTION

In the studies of various procedures designed for the revascularization of myocardium, experimental researches have been carried out usually on normal hearts or on the hearts whose main branch of the coronary artery was ligated.[20)26)] However, inter- and extra-coronary collatral circulation developing in the normal heart differs possibly from that in the ishemic heart,[13)14)43)] and the acute infarction induced by the one-stage ligature of the coronary artery is by no means the same as coronary insufficiency in human beings. It seems to be quite illogical to use the experimental results on normal hearts in order to comprehend the disease in human beings.

For the purpose of producing experimental coronary insufficiency, many investigators desired to get practical methods for the gradual narrowing of the coronary artery. Of aorta and other larger arteries, successful devices for occluding gradually, so as to allow the collaterals to develop, were reported by several investigators.[4)5)10)11)21)25)40)] Concerning the coronary artery, however, noteworthy contribution had been scanty for a long time because of its anatomical particularities. The coronary arteries are too small to be treated with the methods for larger arteries. Moreover, the situation of the artery on the surface of the pulsating heart precludes intricate handlings and equipages.

The following are the works for the gradual narrowing of the coronary arteries reported up to this time.

BLUM,[6)] THORITON and their co-workers[41)] devised a screw clamp, and PHELPS[35)] another type of clamp self-acting through osmosis. NEUMANN and his associates[32)44)] used stainless steel clip which was held open by absorbable catgut. LITVAK and his associates[13)29)] adopted cylindrical hygroscopic casein plastic. Contrary to these external compression methods, GAGE[15)16)] succeeded in coronary artery occlusion using the thrombogenesis of magnesium alloy.

Though the above-mentioned methods have their respective advantages, yet they are far from ideal, regarding the ability to reproduce the coronary insufficency of human beings.

This report will describe a new and useful method for the gradual narrowing of the coronary arteries, utilizing dicetyl phosphate in a quite unique way.

DICETYL PHOSPHATE AND ITS BIOLOGICAL PROPERTIES

Since the conspicuous biological action of polythene-type of cellophane was discovered by PAGE,[33] who described it to be fibroblastic, and since the action of the cellophane was found to be exerted by dicetyl phosphate, the cellophane or dicetyl phosphate has been used in the projects of the gradual narrowing of the various luminal organs in anticipation of the cicatricial shrinkage.[1)2)3)30)46)] Considering the communicated fibroblastic properties of polythene or dicetyl phosphate, it was quite natural that several investigators[3)32)42)] used these materials for the gradual occlusion of the coronary arteries.

However, the experimental results with these materials seemed to have fallen short of their expectations, that is, the narrowing of the coronary arteries was not gained, or even if gained, was unreliable presumably owing to its simple wrapping of the arteries.

For the purpose of reinvestigating the biological properties of dicetyl phosohate, animal experiments were undertaken by using dogs and rabbits. The compound was synthesized in our laboratory with the help of Prof. S. KUNICHIKA and Dr. S. OKA of the Institute for Chemical Research, Kyoto University.

As a result of our experiments, it was confirmed that the biological property of dicetyl phosphate was rather granuloma-producing than fibroblastic. When a strip of spongel containing dicetyl phosphate was inserted into the tissues of dogs or rabbits, wandering cells accumulated gradually around the crystals of dicetyl phosphate. Eventually, the accumulation of wandering cells became an enormous, elastic hard granuloma about 3 weeks later (Fig. 1 a). However, its main cellular constituent was macrophages (Fig. 2) instead of fibrocytes or collagenous fibres, suggesting an unlikely tendency to cicatricial shrinkage. The granuloma, after

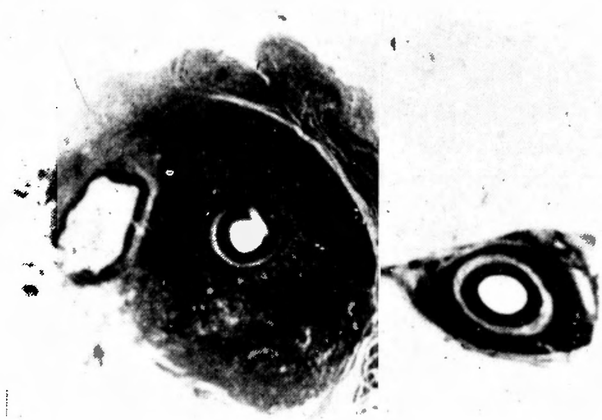

Fig. 1

a

Granuloma produced by DCPS around right femoral artery of rabbit. ×4 hematoxylin and eosin stain.

b

Limited cellular infiltration around left femoral artery of same rabbit, wrapped by simple spongel. ×4 hematoxylin and eosin stain.

maintained for about 3 months with little changes in its volume and consistency, began to be absorbed slowly, and became a meagre scar tissue 6 months later.

The simple spongel which was used as the sustaining substance produced no granuloma but limited cellular infiltration (Fig. 1 b).

After these preliminary experiments, it was concluded that dicetyl phosphate was not useful for occluding the artery so long as cicatricial shrinkage was aimed at.

In the following experiments, there-fore, dicetyl phosphate was used by reason of its granuloma-producing property with a device for multiplying the expansile power of the growing granuloma to compress the coronary arteries.

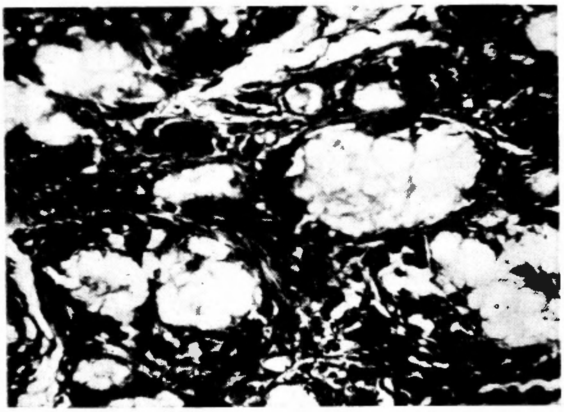

Fig. 2 Macrophages accumulating around dicetyl phosphate crystals which are seen as white leaflets. ×400 hematoxylin and eosin stain.

EXPERIMENTAL METHOD

Preparation of Dicetyl Phosphate Spongel

According to the description of BERNAN,[3] dicetyl phosphate (DCP) was synthesized from cetyl alcohol and phosphorus pentoxide under the anhydrous condition and purified by several recrystallizations by using ethanol acetone and ethanol. The resulting dicetyl phosphate is a white powder, insoluble in water but soluble in hot ethanol, the melting point being 72.0 to 72.5 degrees.

In order to prepare dicetyl phosphate spongel (DCPS), 0.5 gm. of DCP was dissolved in 12 ml. of ethanol by heating, and the resulting solution was poured diffusely into 10 ml. of spongel (gelatine sponge manufactured by YAMANOUCHI Co. Japan). The spongel, soaked with ethanol solution of DCP, was kept in a desicator for several days to evaporate the ethanol. After that, DCP was recrystallized diffusely throughout the spongel. Every process mentioned above was done under the aseptic condition.

As the result of comparative experiments with various amounts of the content of DCP, it was demonstrated that the spongel containing 0.05 gm. of DCP per ml. was the most effective. Consequently, every DCPS used in the following experiments was the one which contained such amount of DCP.

Healthy adult mongrel dogs were anesthetized with the intravenous administration of thiopental sodium so that the wink reflex might scarcely remain. The animals were intubated, and a left thoracotomy was performed in the fourth intercostal space. Respiration was maintained by the intermittent positive pressure breathing by using the room air.

The pericardium was incised anterior to the phrenic nerve. The proximal part of the anterior descending branch of the left coronary artery was isolated from the surrounding tissues at two sites each 5 to 7 mm. apart from other. Two pieces of stainless steel wire, 0.2 mm. in diameter, were passed beneath the artery at both sites (Fig. 3 a). DCPS, 10 by 8 mm. in area, 5 mm. in thickness, soaked in saline solution and flattened by finger press, was placed upon the vessel. An aluminium plate, as large as the DCPS, pierced by several holes, was overlapped on the spongel (Fig. 3 b). Thereafter, each end of the wire was drawn up through the spongel and the corresponding holes of the aluminium plate, and tied with each other to make a loop (Fig. 3 c and d). The resulting two loops of stainless steel

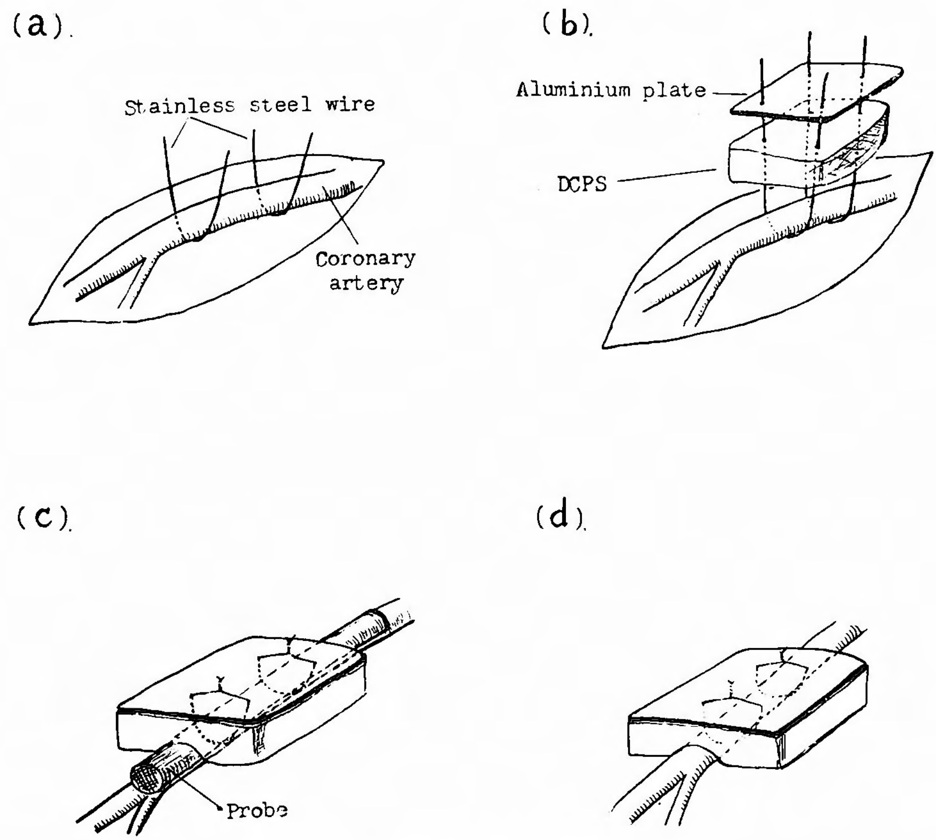

Fig. 3 Technique for application of DCPS.

a) Stainless steel wire is passed beneath coronary artery at two sites.
b) Each end of the wire is drawn up through DCPS and corresponding holes of aluminium plate.
c) Ends of wire are tied with each other tightly over probe held parallel to artery.
d) Probe taken away. Loops of wire become able to surround artery just sufficiently.

wire, each 5 to 7 mm. apart from the other, came to be equipped with an aluminium plate and contain the artery and the DCPS within them. At the time of making the loops, it was advantageous to hold a probe as big as the artery parallel to the vessel in order to avoid the artery being occluded at the first step (Fig. 3 c). Immediately after the wires were tied tightly over the probe, the probe was taken away. Thus the loops of wire were able to surround the artery just sufficiently (Fig. 4).

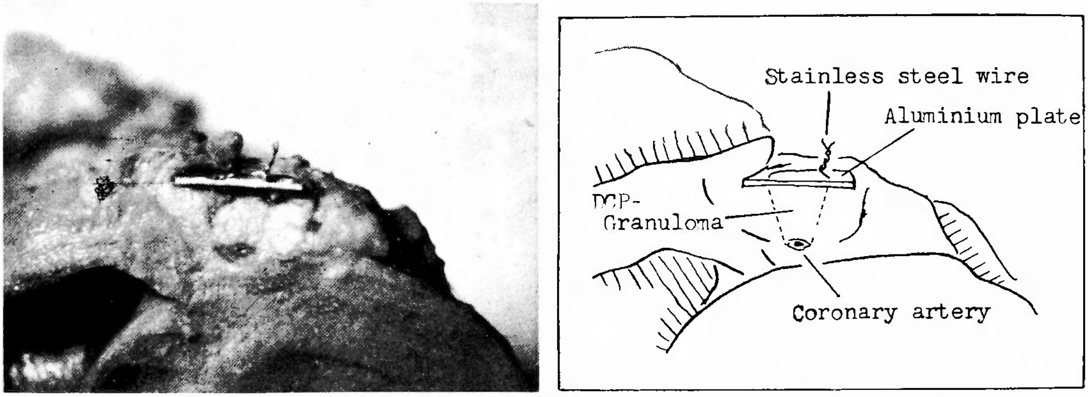

Fig. 4 a-b. Photograph of cross section of specimen, showing coronary artery flattened beneath well developed granuloma.

Usually two loops were made in a procedure.

In the majority, the procedure was executed with the anterior descending branch, but in 3 cases with the circumflex branch and in the other 3 both with the anterior descending and circumflex.

The pericardium was loosely approximated with several interrupted sutures, and the chest was closed routinely after the reinflation of the lung. Penicillin was injected intramuscularily in order to prevent postoperative infection.

Comparative Experiments

For comparative studies, two experiments were performed as follows.

1) To examine the suspicious hoisting effect of the spongel, which was the sustaining substance of DCP, a simple spongel was used in place of DCPS under the same operative procedures and postoperative follow-ups.

2) To examine the influences of mechanical injuries due to the operative procedures upon myocardial electricity, the anterior descending branch was dissected in the same manner as was previously described, then the pericardium and chest were closed without further manipulations. Postoperative electrocardiographic tracings were taken equally.

Investigations were carried out from the following points of view.

1) Electrocardiographic investigations: Serial electrocardiographic tracings were taken without anesthesia every 2 to 3 days during the first 10 days and every 5 to 7 days thereafter. The animals were placed pronely on a sheet of canvas which had 4 holes to bring out their legs and fix them. In this way, the posture at tracing could be maintained in the same physiologic position every time. The electrodes were inserted subcutaneously at the proximal portion of 4 limbs. These actions were done without difficulty by training the animals.

2) Anoxemia tests: Anoxemia tests were carried out 32 to 53 days after the operation under general anesthesia with thiopental sodium. The anesthesia was controlled so as to keep the respiration rate in about 20 per minute and to maintain scarcely recognizable wink reflex.

A gas mixture of 10 per cent oxygen and 90 per cent nitrogen was administered for 20 minutes through a mouth-piece connected with an endotracheal tube without rebreathing. Electrocardiographic tracings were taken just before and every 5 minutes after the administration of the gas mixture was commenced. In addition, arterial blood samples for the estimation of the oxygen content were drawn from the femoral artery just before and 15 minutes after the outset of the test.

3) Schlesinger injection studies: According to the description of Schlesinger,[37] the coronary arteries were injected with Schlesinger,s mass under the pressure of 150 mm, Hg, and X-ray pictures were taken after the dissection of the heart.

As the aluminium plate is translucent to X-ray, it was advantageous to investigate the site of narrowing.

4) Histopathological studies: Immediately after the completion of these angiographic studies, the hearts were fixed in 10 per cent formaline solution. Gross and microscopic examinations were carried out in regard to the vascular and myocardial changes. Particularily the histological examinations of the treated sites of the coronary arteries were undertaken by serial sections along the course of the arteries in order to disclose the appearances of arterial narrowing accurately.

RESULTS

Out of thirty-two dogs subjected to this procedure, 5 were sacrificed 3 to 12 days after the operation. Any of the 5, examined in these early periods, showed no constriction of the vessels.

Of the other 27 operated dogs, which were sacrificed 16 to 56 days after the operation, gross and microscopic or radiographic examinations demonstrated the marked constriction (the cross section area reduced to less than 25 per cent of the original area) in 12, the moderate (the cross section area reduced to 25 to 50 per cent of the original area) in 7, the slight (constriction observed clearly but the cross section area not reduced to less than 50 per cent of the original area) in 3, and scarcely observable or no constriction in 5. The results are given in Table I.

Table 1. Results of Experiments with Gradual Narrowing of Coronary Artery in Dogs

Dog No.	Duration of Observation	Site of Narrowing	Intensity of Narrowing (Additional Changes of Artery)	Electrocardiographic Findings in Follow-up Study (in Lead aVF)	Pathological Findings in Myocardium
1.	40 days	Ant. desc. branch	(卌) Thrombus formation		Infarction (transmural)
2.	47 days	Ant. desc. branch	(卄)	Depression of ST segment	Pyknosis, Vacuolization
3.	35 days	Ant. desc. branch	(+)	Elevation of ST seg. with inversion of T wave	Pyknosis, Vacuolization

4.	56 days	Ant. desc. branch	(卄) Destruction of arterial wall with intraluminal invasion by fibrous tissue	Elevation of ST seg. with inversion of T wave	Pyknosis, Vacuolization
5.	45 days	Circumflex branch	(−)	Depression of ST segment	Pyknosis, Vacuolization (slight)
6.	53 days	Circumflex branch	(卅)	Ventricular tachycardia on 10th postoperative day	Infarction (transmural)
7.	28 days	Circumflex branch	(卅)	Elevation of ST seg. with inversion of T wave	No findings
8.	52 days	Ant. desc. branch	(卄) Intimal proliferation	Elevation of ST seg.	Infarction (transmural)
9.	40 days	Ant. desc. branch	(卅) Destruction of arterial wall with intraluminal invasion by fibrous tissue	Elevation of ST seg. with inversion of T wave	Pyknosis, Vacuolization
10.	46 days	Ant. desc. branch	(卅)	Inversion of T wave	Patch-like fibrosis
11.	41 days	Ant. desc. branch	(卅)	Depression of ST seg.	Pyknosis, Vacuolization
12.	51 days	Ant. desc. branch	(−)	Biphasic T wave	No findings
13.	32 days	Ant. desc. branch	(卅) Thrombus formation and destruction of arterial wall	Ventricular premature contraction (20th postoperative day)	Patch-like fibrosis
14.	53 days	Ant. desc. branch	(−)	No findings	No findings
15.	53 days	Ant. desc. branch	(卅) Intimal proliferation	Inversion of T wave	Patch-like fibrosis
16.	16 days	Ant. desc. branch	(卅) Intimal proliferation	Increase in amplitude of T wave	No findings
17.	33 days	Ant. desc. branch	(卅) Thrombus formation	Depression of ST seg.	Pyknosis, Vacuolization (slight)
18.	47 days	Ant. desc. branch	(卅)	Depression of ST seg.	Patch-like fibrosis
19.	36 days	Ant. desc. branch	(卄)	Ventricular premature contraction (10th postoperative day)	Patch-like fibrosis
20.	38 days	Ant. desc. branch	(卅) Thrombus formation		Pyknosis, Vacuolization
21.	38 days	Ant. desc. branch	(+) Intimal proliferation		Patch-like fibrosis
22.	39 days	Ant. desc. branch	(+)		No findings
23.	30 days	Ant. desc. branch	(−)		No findings
24.	38 days	Ant. desc. branch	(卄)		Patch-like fibrosis
25.	35 days	Ant. desc. branch and circumflex branch	(卄)	Ventricular premature contraction from varying foci (9th postoperative day)	Infarction (subendocardial)

26.	39 days	Ant. desc. branch and circumflex branch	(−)		No findings
27.	37 days	Ant. desc. branch and circumflex branch	(+)	Intimal proliferation	Pyknosis, Vacuolization
28.	12 days	Ant. desc. branch	(±)		No findings
29.	7 days	Ant. desc. branch	(−)		N ofindings
30.	6 days	Ant. desc. branch	(±)		Pericarditis
31.	8 days	Ant. desc. branch	(−)		No findings
32.	3 days	Ant. desc. branch	(−)		No findings

Note: (#)···Constriction marked, the cross section area reduced to less than 25 per cent of the original area; (+)···Constriction moderate, the cross section area reduced to less than 50 per cent of the original area; (+)···Constriction slight, the cross section area not reduced to less than 50 per cent of the original area; (±)···Constriction scarcely observable; (−)···Not constricted.

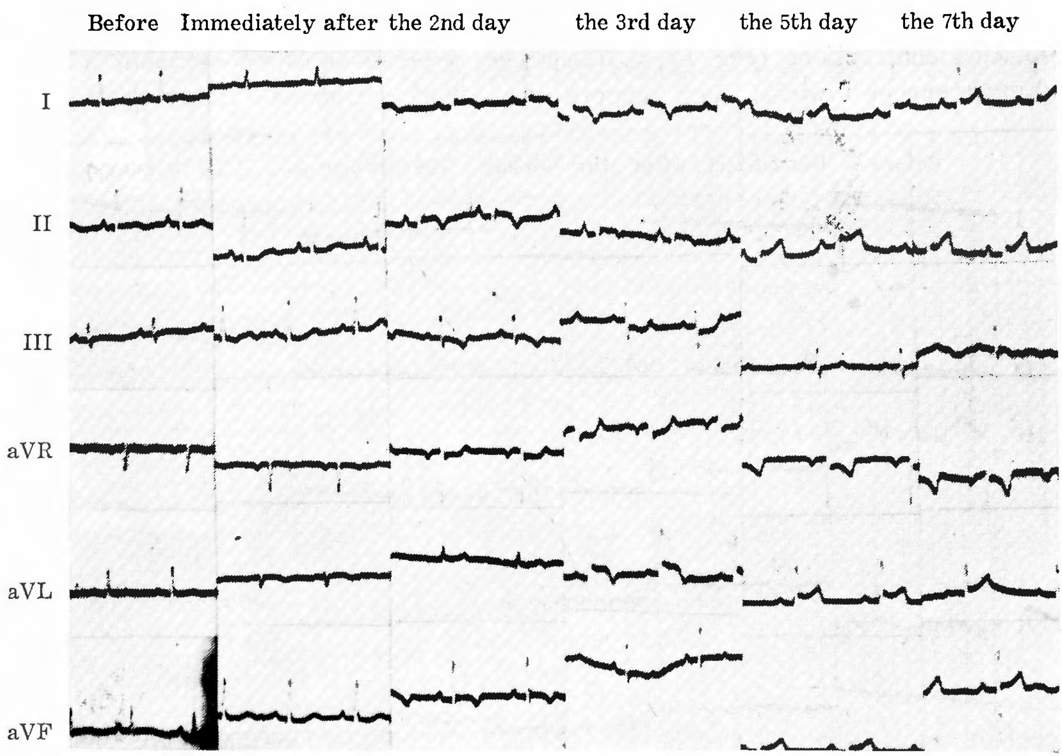

Fig. 5 Electrocardiographic follow-up. Comparative experiment II

(1) Electrocardiographic studies

In the prone position used at tracings in this experiment, lead aVR showed rSr' or rS pattern and lead aVF qRs constantly. Lead aVF was appointed to an indicator of the electrocardiographic investigation, because the lead was thought to be faced always to the epicardial surface of the left ventricle.

In all of 20 explored animals, preoperative tracings demonstrated that ST segment in lead aVF was isoelectric and T wave upward, excepting 4 animals, in which the depression of ST segment within the range of 1.0 mm or biphasic T wave was observed.

Sinus arrhythmia was not considered to be abnormal, because it was usually observed in healthy dogs.[24)34)]

In the majority, depressions or elevations of ST segment and inversions of T wave were recorded in the immediate postoperative periods. As the same findings were observed also in the comparative experiments I and II (Fig. 5), these should be considered not to be originated from coronary artery narrowing but from the direct influence of the operation upon myocardial electricity, that is, possibly from mechanical injuries due to operative procedures, from myocardial anoxemia caused by incomplete reexpansion of the lung or from postoperative pericarditis. In any way, as these initial electrocardiographic findings usually disappeared within a week, they were ruled out from the following descriptions.

Adequate postoperative electrocardiographic follow-ups were carried out on 19 animals, and 16 of them showed several significant findings, including ventricular premature contractions (Fig. 6), elevations or depressions of ST segment (Fig. 7) and inversions of T waves, each suggesting myocardial ishemia. These electrocardio-

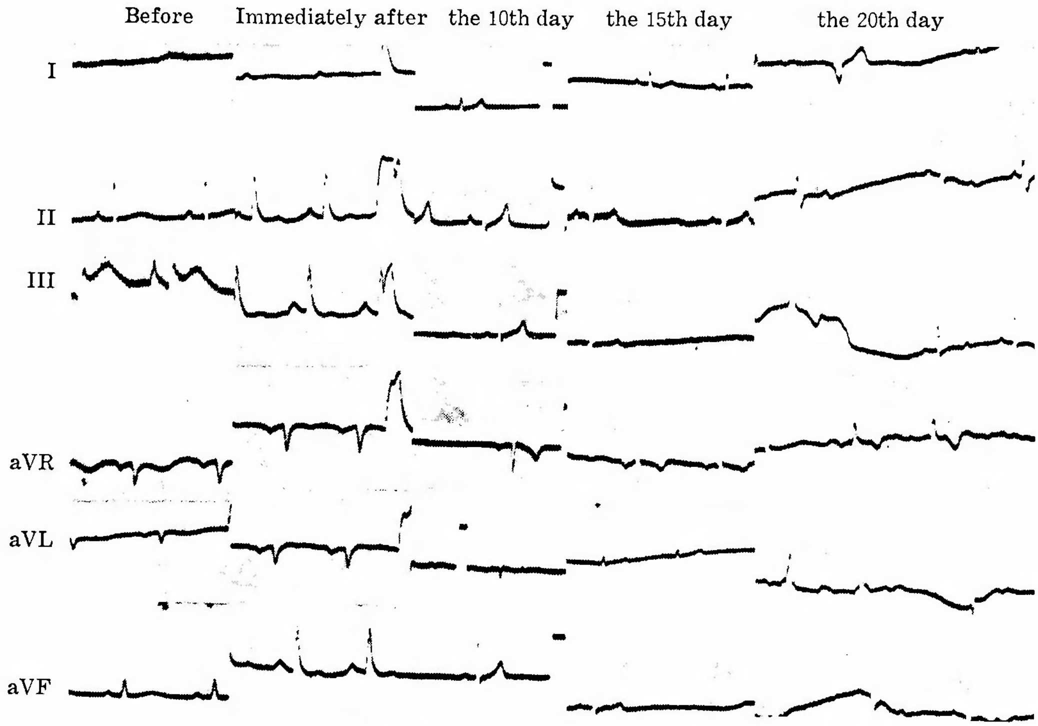

Fig. 6 Electrocardiographic follow-up. No. 13

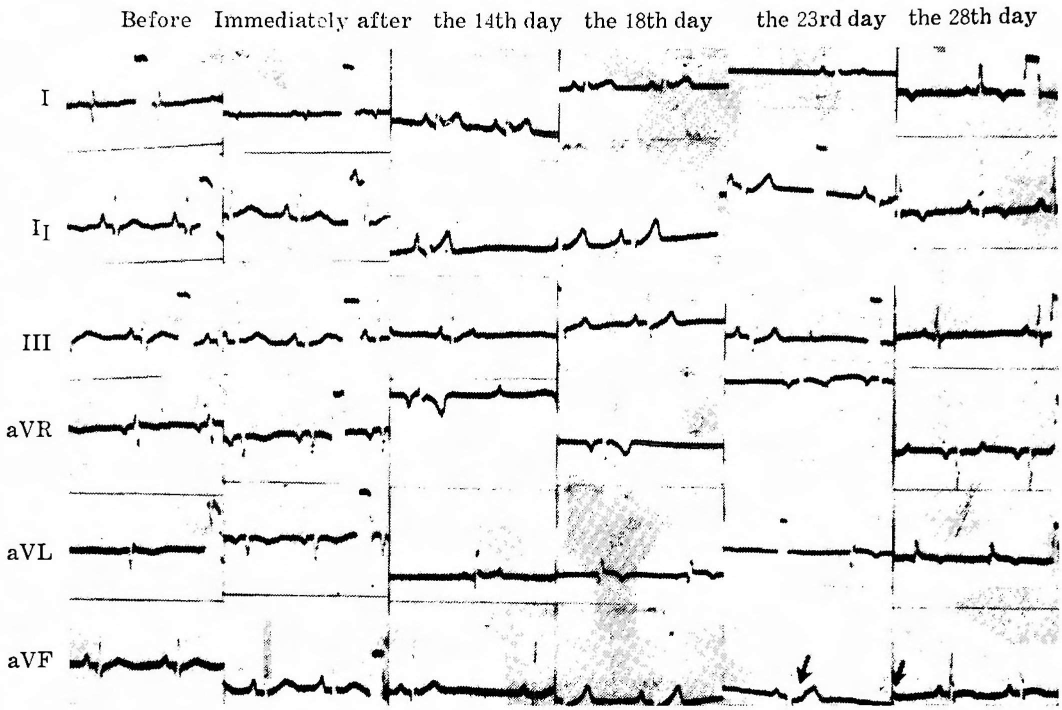

Fig. 7 Electrocardiographic follow-up. No. 18

Table 2. Summary of Electrocardiographic Findings in Follow-up Study (in Lead aVF)

Ventricular premature contraction	4
No. 6 ········· on the 10th postoperative day	
No. 13 ········· on the 20th postoperative day	
No. 19 ········· on the 10th postoperative day	
No. 25 ········· on the 9th postoperative day	
Elevation of ST segment with inversed T wave	5
Depression of ST segment	5
Inversion of T wave	2
Biphasic T wave	1
Increase in amplitude of T wave	1
No finding	1

graphic changes usually began to occur in the 3rd or 4th week and continued for 2 to 3 weeks excepting premature contraction.

Three animals which presented no significant findings through follow-up studies, were found to have failed in developing coronary artery narrowing.

The findings in lead aVF are given in Table 2.

(2) Anoxemia tests.

Anoxemia test was performed on 7 animals. In 3 animals, which had failed in developing coronary artery narrowing, no changes were observed in the electrocardiographic pattern during the anoxemia test, though one of them presented suggestive electrocardiographic findings in the postoperative follow-up. In 2 animals, in which a marked narrowing of the coronary artery was found at autopsies and

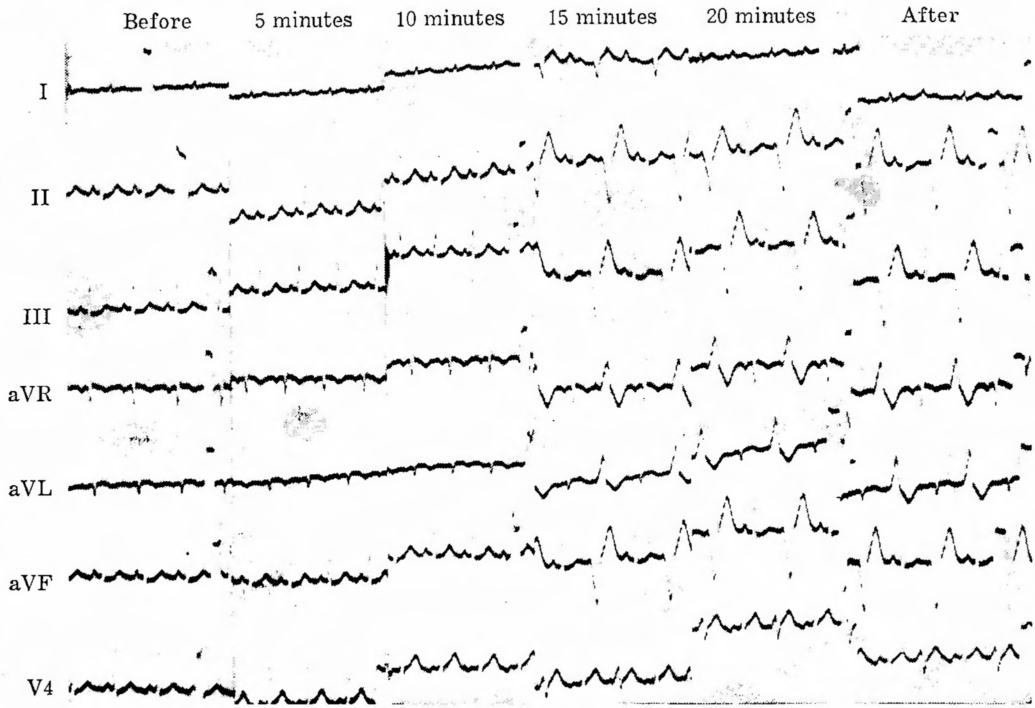

Fig. 8 Anoxemia test. No. 13

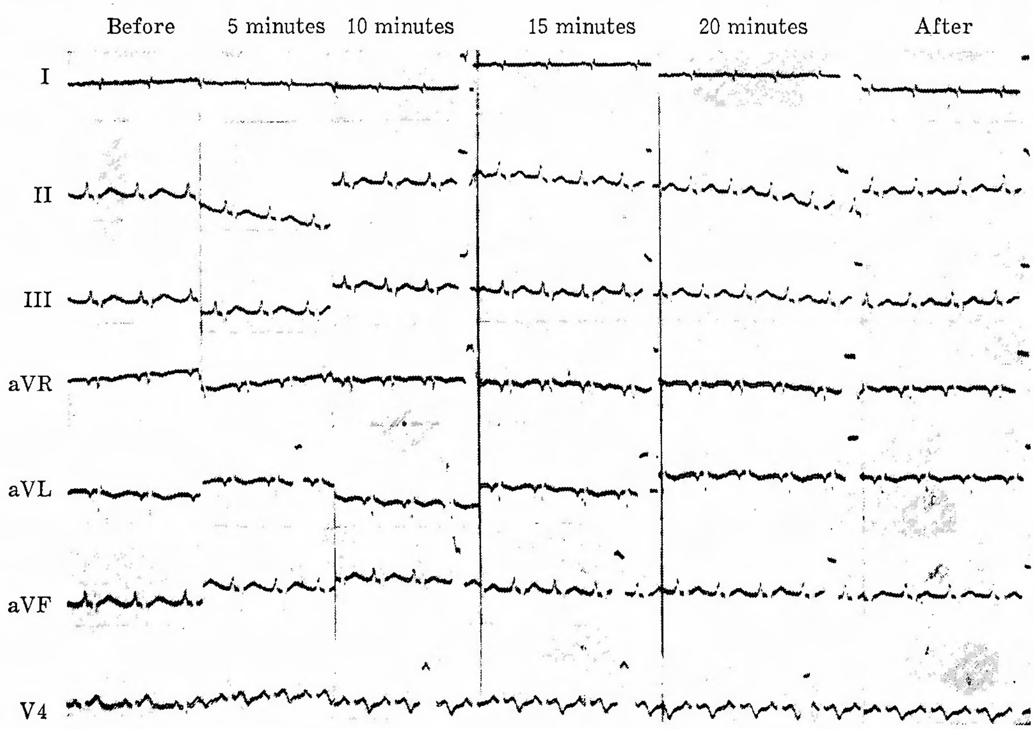

Fig. 9 Anoxemia test. No. 18

ventricular premature contraction had been observed in the postoperative follow-up, bigeminal rhythms were provoked by the inducing anoxia (Fig. 8). In the other 2 animals, which also presented a marked narrowing of the arteries, depressions of ST segment and inversions of T wave in lead V4 were observed during the tests (Fig. 9).

(3) Schlesinger injection studies.

In 5 animals which were sacrificed 3, 6, 7, 8 and 12 days after the operation respectively, coronary narrowing was scarcely or not observed by radiography.

On the other hand, the narrowing was evidently observed in those sacrificed from 16 to 56 days after the operation, although its intensity was not always consistent (Fig, 10 and 11).

Fig. 10 Coronary angiogram (No. 16), showing site of narrowing with arrow.

Fig. 11 Coronary angiogram (No. 11), showing site of narrowing. Stainless steel wire strangled coronary artery, hoisting it upwards from surface of heart.

Radiographic examinations revealed that the stainless steel wire strangeled the artery, hoisting it upwards from the surface of the heart, so as to make kink at the situation. The circumflex branch was usually found to be less markedly narrowed than the anterior descending branch probably by reason of its loose attachment to the surface of the heart.

(4) Gross and microscopic findings of the narrowed coronary arteries.

In order to examine the treated site of the coronary arteries, the aluminium plates were removed after the wire loops had been cut, and cross sections were made at right angles to the arteries close by the wire.

In the animals, sacrificed up to the 12th postoperative day, coronary artery narrowing was not found with the naked eye.

In the animals sacrificed 16 to 56 days after the operations, the arteries were found to have been narrowed more or less markedly. When the vessels had been markedly narrowed, their cross section looked linear or sometimes spot-like, compressed under a well developed granuloma.

Microscopically, the coronary arteries were patent and looked round in their cross section up to the 12th postoperative day, although the arterial wall in 2 animals undulated slightly. The circumferential tissue was edematous and infiltrated mainly by polynuclear leucocytes, afterwards by fibrocytes, while the overlying DCPS was scarcely invaded by wandering cells up to this time. The arteries were not detached from the surface of the heart.

In 22 out of 27 animals, sacrificed 16 to 56 days after the operation, the coronary arteries had been narrowed more or less markedly. Generally speaking, the DCPS, having been laid between the aluminium plate and the coronary arteries, had turned into well developed granulomas mainly composed of macrophages.

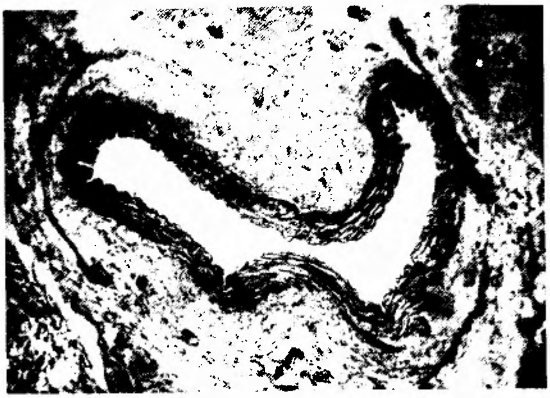

Fig. 12 Photomicrograph of narrowed coronary artery. Folding of arterial wall with surrounding proliferation of fibrous connective tissue (No. 7). ×50 hematoxylin and eosin stain.

In serial sections, the arteries were found to be detached from the surface of the heart, associated with their walls being folded and consequent narrowing of the lumina (Fig. 12), as they approached nearer the wire. A marked proliferation of fibrous connective tissues surrounded the arteries. These occluding changes were confined only within 1 to 2 mm, in distance (Fig. 13a and b).

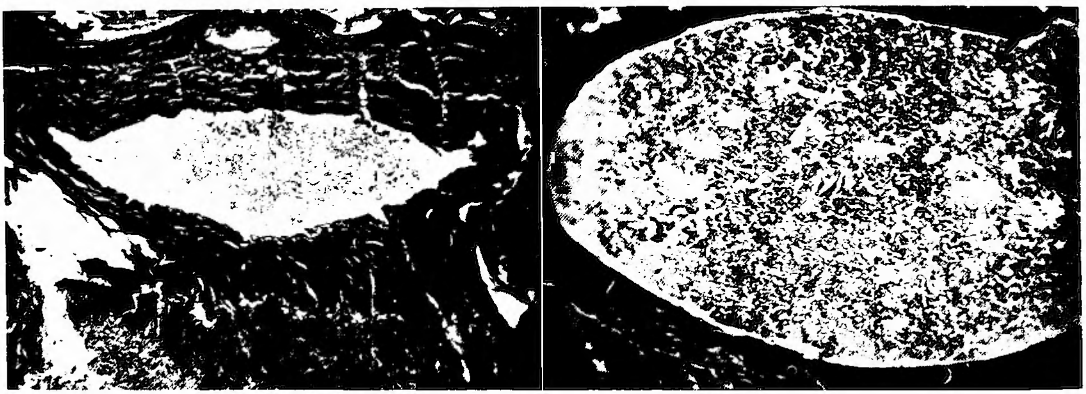

Fig. 13

a) Photomicrograph of narrowed coronary artery (No. 11), showing invasion of arterial wall with fibrous connective tissue. ×50 HEIDENHAIN's azan stain.

b) Two mm upstream to wire portion which illustrated at left photograph. SCHLESINGER's mass filling arterial lumen. ×50 HEIDENHAIN's azan stain.

In the most narrowed portion, the surrounding fibrous connective tissues had invaded the tunica media of the arterial walls (Fig. 13 a), and moreover, in 3 dogs, poured into the lumina of the arteries through the portion in which all the layers of arterial wall had been destroyed mechanically by wire strangulation (Fig. 14 and 15).

Fig. 14 Photomicrograph of narrowed coronary artery (No. 9), arterial wall being destroyed at its bottom and intimal proliferation. ×50 hematoxylin and eosin stain.

Fig. 15 Photomicrograph of narrowed coronary artery (No. 13). Fibrous connective tissue poured into arterial lumen through ruptured portion of wall. Lumen filled with immature connective tissues withholding several holes. ×50 hematoxylin and eosin stain.

Intimal proliferation was found in 7 animals. In an animal, the internal elastic membrane had been ruptured at several portions, associated with the lumen filled with the immature connective tissue, withholding several holes through which blood circulation was partially maintained (Fig. 15). That was thought to be the early state of recanalization of thrombosis. On the other hand, organized thrombi attached to the arterial wall were found in 3 animals (Fig. 16 and 17).

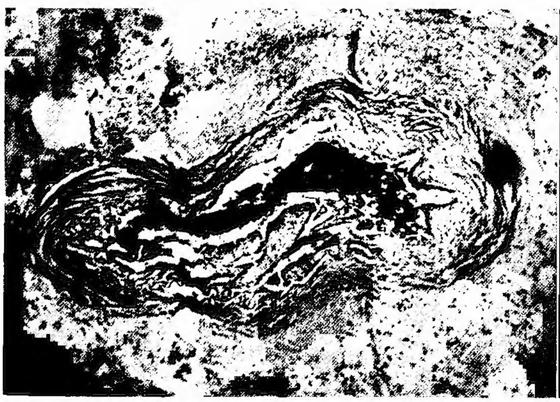

Fig. 16 Photomicrograph of narrowed coronary artery, showing ruptured internal elastic membrane and organized thrombus. ×50 hematoxylin and eosinstain.

Fig. 17 Photomicrograph of narrowed coronary artery, showing the organized thrombus attached to the arterial wall. ×50 hematoxylin and eosin stain.

In 4 out of 5 animals in which arterial narrowing was not found, granulomas had poorly developed and changed into abcess, associated with insignificant fibrocollagenous reaction. The remaining one was that in which circumflex branch was dealt with.

(5) Microscopic findings in myocardium.

Extensive myocardial infarction discernible to the naked eye was found only in 3 out of 27 dogs. All of these 3 dogs showed a marked narrowing of the coronary artery, accompanied with thrombus formation in one of them. A narrowing of rapid progression might have taken place in the early period.

The most interesting finding encountered in our experiments appeared to be patch-like fibrosis in myocardium (Fig. 18). This was found in 6 dogs and

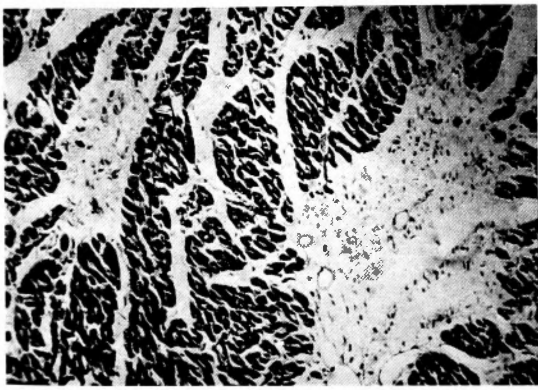

Fig. 18 Photomicrograph of myocardium, showing patch-like fibrosis. ×200 hematoxylin and eosin stain.

Fig. 19 Photomicrograph of myocardium, showing vacuolization of muscle fibres. Some fibres showing complete replacement with vacuoles in middle and middle right. ×600 hematoxylin and eosin stain.

usually dominant in the inner layers of the anterior walls of the left ventricles supplied with blood from the treated coronary arteries. Degenerated muscular fragments with pyknosis were occasionally found. All the animals which presented these findings were found to have succeeded in producing a severe narrowing of the coronary arteries. This patch-like fibrosis resulted presumably from myocardial focal necrosis.

Concerning another point of observation, vacuolization and pyknosis in muscle fibres (Fig. 19) associated with interstitial edema were observed in the portion supplied with blood from the narrowed coronary arteries.

In the portions where these degenerative myocardial changes were not so marked, the dilatation of interstitial capillaries was found frequently.

However, there was an animal (No. 16), in which myocardial changes were scarcely observed in spite of the marked narrowing of the coronary artery.

REPRESENTATION OF SEVERAL CASES

No. 4 : DCPS was applied to the anterior descending branch of the left coronary artery with a piece of stainless steel wire. Electrocardiographically T wave in lead II, III and aVF became biphasic or flattened after the 26th postoperative day. Sacrificed on the 56th postoperative day, the anterior descending branch was found to be compressed beneath the granuloma, its wall destroyed by fibrous connective tissue invasion. Myocardium presented severe vacuolization and pyknosis of muscle fibres, some fibres showing complete replacement with vacuoles (Fig. 19). The proliferation of the connective tissue in myocardium was not observed.

No. 9: DCPS was applied to the anterior descending branch with 2 pieces of wire. Electrocardiographically, T wave in lead aVF became flattened after the 19th day, then turned biphasic with the elevation of ST segment on the 43rd day. Sacrificed on the 48th postoperative day, the artery looked linear in the cross section beneath the well developed granuloma.

Histologically, the destruction of the arterial wall, fibrous connective tissue invasion into the lumen, and remarkable intimal proliferation were found at the treated site (Fig. 14). Myocardium was found to be dotted with muscle fibres which were stained stronger by eosin and revealed indistinct striation, mainly in the anterior wall of the left ventricle (Fig. 20). Vacuolization and pyknosis were also marked.

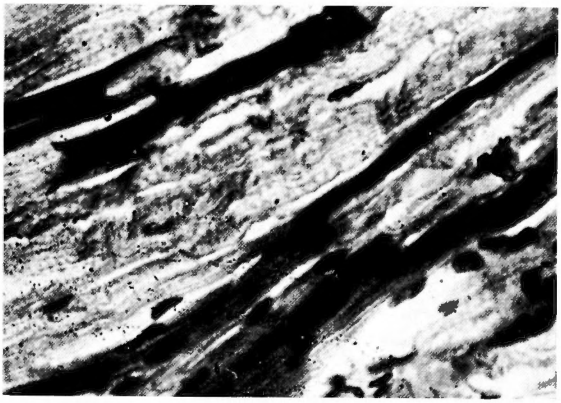

Fig. 20 Photomicrograph of myocardium, some muscle fibres showing stronger eosin-staining and indistinct striation. ×600 hematoxylin and eosin stain.

It was thought in these 2 animals that coronary artery narrowing had advanced with the invasion of arterial lumina by fibrous connective tissues at the site where the arterial wall was mechanically destroyed by the continuous compression with stainless steel wire.

No. 11: DCPS was applied to the anterior descending branch with 2 pieces of wire. Electrocardiographically, ST segment depression in lead aVF was observed from the 31st postoperative day, and concurrently the exercise ability of the dog was so markedly reduced that the dog became unable to run even 500 meters. On the 41st postoperative day, the dog suddenly expired during the intravenous injection of 1.5 per cent solution of thiopental sodium for anoxemia test. Postmortem injection study and microscopic examination revealed that the anterior descending branch had been severely constricted (Fig. 11). Histologically, the artery was severely narrowed at the site of wire, hoisted upwards from the heart surface, with the proliferation of the fibrous connective tissue and its invasion into the tunica media of the arterial wall (Fig. 13 a). The remarkable vacuolization and pyknosis of muscle fibres were found in the apical portion of the heart.

It was supposed from these observations that the animal was killed by ventricular fibrillation due to the "Trigger Mechanism" of Beck, that is, respiratory depression caused from thiopental sodium anesthesia had provoked ventricular fibrillation through the aggravation of myocardial anoxemia or oxygenation difference which had already reached its critical point with the progression of coronary artery narrowing.

No. 13: DCPS was applied to the anterior descending branch with 2 pieces of wire. Postoperative electrocardiographic tracings showed the normal pattern up to the 14th day. On the 20th postoperative day, ventricular premature contraction occurred and continued for less than 5 days (Fig. 6). ST segment depressions in lead aVF remained thereafter. On anoxemia test, performed on the 31st postoperative day, bigeminal rhythm was provoked ten minutes after the administration of gas mixture was commenced (Fig. 8). Postmortem injection study showed a severe narrowing of the artery. Histological examination demonstrated that the fibrous connective tissues around the treated site of the artery had invaded the arterial wall and, furthermore, poured into the arterial lumen through ruptured internal elastic membranes. The lumen had been almost entirely filled with immature connective tissues, withholding several holes for maintaining blood circulation (Fig. 15). In myocardium, typical patch-like fibrosis with pyknosis and vacuolization of muscle fibres were observed (Fig. 18 and 19).

It was presumed from these observations that coronary artery narrowing had progressed rapidly with thrombus formation about 20 days after the operation and broken the equilibrium between oxygen needs and its supplies in myocardium with the resultant ventricular premature contraction and patch-like fibrosis.

No. 18: For the purpose of strengthening the hoisting effect of stainless steel wire, the anterior descending branch was fixed to myocardium with a silk suture at the site of the wire; the silk suture was laid down so as to cross the wire and to make a slit composed of silk and wire through which the artery passed. Electrocardiographic tracings showed ST segment depressions in lead II, III and aVF from the 18th postoperative day (Fig. 7). On anoxemia test, performed on the 47th day, complete inversions of upward T wave in lead V4 were observed 15 minutes after the onset of the test (Fig. 9). Schlesinger injection study and histological examination demonstrated that the coronary artery had not been hoisted by stainless steel wire, as intended, but that the arterial wall had been folded remarkably with the consequent narrowing of the lumen. The surrounding proliferation of connective tissues was prominent. Patch-like fibrosis and pyknosis in myocardium were found in the subendocardial region of the anterior wall of the left ventricle.

No. 12: This is an example of unsuccessful cases. Two pieces of wire were used for applying the DCPS to the anterior descending branch. Postoperatively no significant findings were found electrocardiographically up to the 31st day, when T wave in lead II, III and aVF became isoelectric or biphasic, though the downward deflection of the biphasic T wave remained within 1mm in depth. On anoxemia test, performed on the 51st postoperative day, the electrocardiographic pattern was absolutely unchanged during the test. Postmortem injection study and histological examination failed to show arterial narrowing. Granuloma had developed poorly. Myocardial degenerative changes were not found.

As the animal had been lean, suffering from diarrhea and vomitting before and for about 2 weeks after the operation, it was supposed that undernourishment had caused the granuloma not to develop largely enough to occlude the coronary artery.

DISCUSSION

Although many investigators had tried to use dicetyl phosphate or a type of cellophane for the purpose of the gradual occlusion of the coronary artery, yet all the trials seem to have been futile up to this time.

As described before, many investigators unreasonably expected that the coronary arteries would be occluded through the cicatricial shrinkage of the fibrous connective tissue produced by dicetyl phosphate or the cellophane. Actually, as illustrated by our own investigations, dicetyl phosphate does not yield enough fibrocollagenous tissues to constrict the arteries, but produces voluminous granulation tissue. That is a reason for the futility.

On the other hand, it seems equally illogical to expect the growing granuloma may compress the coronary artery directly when produced within a metal cylinder.

Capillary pressure is always lower than arterial pressure in the same being, and accordingly, it is always the capillary vessels of the granuloma and not the arterial vessel that are obliterated when the growing granuloma and artery are put in such a limited space as a metal cylinder. In other words, in those circumstances, the granuloma does not grow enough to compress the arterial vessel, because the pressure atrophy of the granuloma is brought about before the arterial vessel is compressed.

As a matter of fact, NEUMANN et al.[32)] reported that the injection of dicetyl phosphate into the adventitia of the coronary artery, combined with the encirclement of the artery with a 1 cm metal band, had not brought forth a sufficient fibrous tissue to cause the constriction of the artery.

In the device introduced here, it was intended to utilize the expansile power of the growing granuloma produced by dicetyl phosphate efficiently. As the aluminium plate and stainless steel wire are able to be considered as a rigid body, the expansile power of a growing granuloma received at the inner surface of the aluminium plate is transmitted wholly to any point of the wire and, of course, to the limited portion where the wire comes in contact with the arterial wall. At that portion the transmitted expansile power is able to exert a hundredfold or more multiplied strength per area upon the arterial wall and to hoist and kink the coronary artery against the arterial pressure and all the resistances of the arterial wall. In this way, the coronary artery is bent and narrowed at the wire portion, because the artery is hoisted with the wire while it is left to be fixed under epicardium upstream and downstream to the wire portion. In these conditions, the more markedly and the more limitedly the coronary artery is hoisted, the sharper the bending of the artery and the severer the resultant narrowing becomes. Accordingly, it seems more advantageous for the development of narrowing to confine the extent of dissection of the artery within a possibly short distance when the vessel is dissected for encirclement with wire.

With the circumflex branch of the left coronary artery, marked narrowing had not been obtained as compared with the anterior descending branch, because it was usually lying within loose subepicardial fat which resulted in the less efficient hoisting of the circumflex branch. In order to get a more efficient hoisting of the artery, it seems advantageous to attach the vessel with silk suture to the myocardial surface just near the wire portion. This attachment of the artery was done in No. 18 and No. 19 with excellent results.

After all, as the first step to success by this method is to make a huge and solid granuloma, malnutrition or local infection with the resultant undergrown granuloma appears to be the main cause of failure.

While such morphological changes of the coronary arteries as hoisting and bending are the primary factor in the development of the narrowing of the artery, it is followed secondarily by organic changes in and around the wall of the artery. Fibrous connective tissues proliferate abundantly around the coronary artery, and furthermore, they invade the wall and lumen of the vessel through the portion where the arterial wall has been destroyed by continuous compression with wire. Intimal proliferation and thrombus formation can develop concomitantly. In this way, the coronary artery narrowing which has developed at first merely through mechanical compression, appears to be maintained or advanced by these secondary organic changes of the arterial wall.

The term of "Coronary Insufficiency" indicates the condition in which there is a disproportion between the oxygen need of the heart and the oxygen supply to the heart. While coronary artery narrowing is usually one of the most important causes of coronary insufficiency, coronary artery narrowing, in itself, is not to be a synonym of coronary insufficiency. When coronary artery narrowing develops more slowly, intercoronary collaterals act and alleviate the disturbance of coronary blood flow, and consequently the state of coronary insufficiency is often not brought about even in case of the complete occlusion of a main branch of coronary arteries.[7]

In our experiments, most of the animals which presented significant findings in the electrocardiographic follow-up, demonstrated a distinct narrowing of the coronary artery, and those with no finding usually did not. However, several cases did not present any significant electrocardiographic finding in spite of the marked narrowing of the coronary artery (No. 11 and No. 16). These seem to be the examples in which the sufficient development of collaterals had compensated for the circulatory disturbances of coronary blood flow. Though these compensated animals had got free of severe myocardial damages, "Coronary Reserve", the margin of safety, must have been decreased so profoundly that once such an extraordinary condition as anoxia or increased cardiac work necessitates total coronary flow increase, the disturbing effect of arterial narrowing[19)38)] possibly becomes distinct, followed by acute coronary insufficiency.

Based on these considerations, anoxemia test[22)28)39)] was performed on several animals. All of the 4 animals, in which postmortem examinations showed a severe narrowing of the coronary arteries, presented remarkable alterations of the electrocardiographic pattern after inducing anoxemia. Contrary to these, none of the other 3 animals which had failed in developing coronary artery narrowing presented any alterations during anoxemia test. It is very interesting that anoxemia test fell into negative in the animal (No. 12) which had shown a suggestive biphasic Twave in lead aVF from the 30th postoperative day in spite of having failed in developing coronary artery narrowing.

We tried the test on the 2 dogs in which the anterior descending branch had been ligated in one stage a month before and infarction curve observed in lead V4. These dogs did not show any alterations of the electrocardiographic pattern during the tests.

These results of anoxemia test seem to suggest that the test is an efficacious diagnostic method also in experimentally produced coronary insufficiency in dogs.

In 6 animals myocardial patch-like fibrosis was found in the territories of the narrowed coronary artery. The narrowing of the artery in these animals was always severe in degrees, associated with distinct electrocardiographic findings.

Nowadays, "Disseminierte Parenchymnekrose" described by BÜCHNER[8)9)] is acknowledged to be the morphological aspect of coronary insufficiency. The patch-like fibrosis in our experiment is thought to be its sequel. When blood supply through the narrowed coronary artery becomes unable to meet the oxygen need of myocardium for a certain duration, which may happen in the time of rapid progression of narrowing or increased cardiac work, myocardial muscles are destroyed irreversibly to form the disseminated necrosis which in turn develops into the patch-like fibrosis. In our experiments the animals were kept in a limited space of the kennel and not forced to exercise. If the animals had been loaded with appropriate exercise, the patch-like fibrosis might have been obtained more frequently.

In the other animals, while they did not show those distinct myocardial findings, vacuolization[31)36)] or pyknosis in muscle fibres was observed in the majority, indicating myocardial hypoxia.

SUMMARY AND CONCLUSIONS

(1) A new method for the gradual narrowing of the coronary artery was described. The coronary artery was constricted gradually with the growing granuloma produced by dicetyl phosphate with the aid of loops composed of an aluminium plate and stainless steel wire.

(2) Any of the five dogs which were subjected to this procedure and sacrificed within 12 days showed no constriction of the coronary artery.

Out of the 27 operated dogs, examined 16 to 56 days after the operation, 12 showed the marked constriction (the cross section area reduced to less than 25 per cent of the original area), 7 the moderate (25 to 50 per cent of the original area), 3 the slight (up to 50 per cent of the original area) and 5 scarcely observable or no constriction.

(3) The narrowing of the coronary artery is brought about at first by the external compression of the vessel with stainless steel wire, then maintained or advanced by the secondary organic changes of the arterial wall, including encirclement and invasion of the arterial wall with the fibrous connective tissue, intimal proliferation and thrombus formation.

(4) Most of the dogs of which the coronary artery was narrowed severely, presented the electrocardiographic findings similar to the clinical picture of coronary insufficiency in human beings. Histological examinations demonstrated patch-like fibrosis, vacuolization and pyknosis of muscle fibres in the portion of myocardium supplied with blood from the narrowed coronary artery. These findings are thought to be suggestive of myocardial ishemia.

(5) The method for producing experimental coronary insufficiency described in this paper, seems to be one of the most excellent to reproduce the clinical pictures of the disease and recommendable because of its easiness in technique and high percentage of success.

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REFERENCES

1) Abbott, O. A.: Clinical Experiences with the Application of Polythene Cellophane upon Aneurysms of the Thoracic Vessels. J. Thorac. Surg., **18**, 435, 1949.
2) Berman, J. K. and Hull, J. E.: Experimental Ascites -- Its Production and Control. Surgery, **32**, 67, 1952.
3) Berman, J. K. and Hull, J. E.: The Treatment of Aneurysms with Fibroblastic Agents; Experimental and Clinical Studies with the Use of Sodium Dicetyl Phosphate. Surg., Gynec. and Obstet., **94**, 543, 1952.
4) Berman, J. K. et al.: Gradual Vascular Occlusion. Surgery, **39**, 399, 1956.
5) Blakemore, A.: Progressive Constrictive Occlusion of the Abdominal Aorta with Wiring and Electrothermic Coagulation. Ann. Surg., **133**, 447, 1951.
6) Blum, L., Schauer, G. and Calef, B.: Gradual Occlusion of a Coronary Artery. Amer. Heart J., **16**, 159, 1938.
7) Blumgart, H. L. and Schlesinger, M. J.: Angina Pectoris, Coronary Failure and Acute Myocardial Infarction; The Role of Coronary Occlusion and Collateral Circulation. J. A. M. A., **116**, 91, 1941.
8) Büchner, F.: Das morphologische Substrat der Angina pectoris im Tier Experiment. Beitr. path. Anat., **92**, 311, 1934.
9) Büchner, F. und Lucadou, W.. Elektrokardiographischen Veränderungen und disseminierte Nekrosen des Herzmuskels bei experimenteller Coronarinsuffizienz. Beitr. path. Anat., **93**, 169, 1934.
10) Clatworthy, H. W. et al.: Coarctation of the Aorta; Its Experimental Production in Dogs, with Special Reference to Technical Methods Capable of Inducing Significant Intraluminal Stenosis. Surgery, **28**, 245, 1950.
11) Cooper, F. W. et al.: The Experimental Production of Gradual Occlusion of Large Arteries with Polythene and Tantalum. Surgery, **25**, 184, 1949.
12) Craig, R. L. and Learnd, B. B.: Patterns of the Anterior Descending Branch of the Left Coronary Artery in the Dog. Amer. Heart J., **48**, 455, 1954.
13) Duchesne, E. R. and Vineberg, A.: An Experimental Study of the Effect of Mechanically Induced Ishemia upon the Mammary Coronary Anastomosis. Surgery, **43**, 837, 1958.
14) Eckstein, R. W. et al.: Effect of Exercise and Coronary Artery Narrowing on Coronary Collateral Circulation. Circulation Reseaech, **5**, 230, 1957.
15) Gage, A. A., Olson, K. C. and Chardack, W. M.: Experimental Coronary Thrombosis in the Dog. Ann. Surg., **143**, 535, 1956.
16) Gage, A. A., Olson, K. C. and Chardack, W. M.: Cardiopericardiopexy; An Experimental Evaluation. Ann. Surg., **147**, 289, 1958.
17) Garamella, J. J. and Hay, L. J.: A Correlative Study of Peripheral Coronary Pressure and

Coronary Arteriography Following Coronary Occlusion. Surg., Gynec. & Obstet., **105**, 89, 1957.
18) Giese, V. W.: Die Anastomosen in Koronarkreislauf bei Koronarsklerose. Deutsch. Med. Wchnschr., **82**, 602, 1957.
19) Gregg, D. E.: Coronary Circulation in Health and Disease. Lea & Febiger. Philadelphia, 1950.
20) Hahn, R. S. and Beck, C. S.: Revascularization of the Heart; A Study of Mortality and Infarcts Following Multiple Coronary Artery Ligation. Circulation, **5**, 801, 1952.
21) Haimovici, H.: Stenosing Arterial Thrombosis; An Experimental Physiopathologic Study. Surgery, **36**, 1075, 1954.
22) Hannon, D. W. et al.: Staged Occlusion of the Coronary Arteries and Studies Under Low Oxygen Tension. J. Thorac. Surg., **32**, 1, 1956.
23) Herrmann, G. R., Marchand, E. J., Greer, G. H. and Hejtmancik, M. R.: Pericarditis Clinical and Laboratory Data of 130 Cases. Amer. Heart J., **43**, 641, 1952.
24) Horwitz, S. A., Spanier, M. R. and Wiggers, H. C.: The Electrocardiogram of the Normal Dog. Proc. Soc. Exp. Biol. & Med., **84**, 121, 1953.
25) Jacobson, J. H. and McAllister, F. F.: A Method for the Controlled Occlusion of Larger Blood Vessels. Ann. Surg., **145**, 334, 1957.
26) Leighninger, D. S.: A Laboratory and Clinical Methods of Evaluation of Operations for Coronary Artery Disease. J. Thorac. Surg., **30**, 397, 1955.
27) Lengyel, L., Caramelli, Z., Monfort, J. and Guerra, J. C.: Initial Electrocardiographic Changes in Experimental Occlusion of the Coronary Artery in Nonanesthetized Dogs with Closed Thorax. Amer. Heart J., **53**, 334, 1957.
28) Levy, R. L. et al.: The "Anoxemia Test" in the Diagnosis of Coronary Insufficiency. Amer. Heart J., **21**, 634, 1941.
29) Litvak, J., Siderides, L. E. and Vineberg, A. M.: The Experimental Production of Coronary Artery Insufficiency and Occlusion. Amer. Heart J., **53**, 505, 1957.
30) Middleman, I. C. and Drey, G.: Cellophane Wrapping of an Abdominal Aortic Aneurysma. Surgery, **29**, 890, 1951.
31) Müller, E und Rotter, W.: Über histologische Veränderungen beim akuten Höhentod. Beitr. path. Anat., **107**, 156, 1942.
32) Neumann, C. G., Moran, R. E., Wedel, J. V., Lord, J. W. and Hinton, J. W.: Reduction of Coronary Artery Blood Flow Preparatory to Revascularization of the Heart via Pedicled Flap of Skin. Plast. & Reconstruct. Surg., **13**, 85, 1954.
33) Page, I. H.: The Production of Persistent Arterial Hypertension by Cellophane Perinephritis. J. A. M. A., **113**, 2046, 1939.
34) Peterson, E. S. et al.: Electrocardiogram of the Beagle Dog. Proc. Soc. Exp. Biol. & Med., **77**, 330, 1951.
35) Phelps, K. R.: A New Clamp for the Gradual Occlusion of Coronary Arteries. Amer. Heart J., **21**, 664, 1941.
36) Pichotka, J.: Tierexperimentelle Untersuchungen zur pathologischen Histologie des akuten Höhentodes. Beitr. path. Anat., **107**, 117, 1942.
37) Schlesinger, M. J.: An Injection plus Dissection Study of Coronary Artery Occlusions and Anastomoses. Amer. Heart J., **15**, 528, 1938.
38) Shipley, R. E. and Gregg, D. E.: The Effect of External Constriction of a Blood Vessel on Blood Flow. Amer. J. Physiol., **141**, 289, 1944.
39) Stewart, H. J. and Carr, H. A.: The Anoxemia Test. Amer. Heart J., **48**, 293, 1954.
40) Stone, P and Lord, J. W.: An Experimental Study of the Thrombogenic Properties of Magnesium and Magnesium-Aluminium Wire in the Dog's Aorta. Surgery, **30**, 987, 1951.
41) Thornton, J. J. and Mautz, F. R.: Experimental Methods for Producing Chronic, Progressive, Coronary Arterial Occlusion. Amer. Heart J. **19**, 404. 1940.
42) Vineberg, A. et al.: Four Years' Clinical Experience with Internal Mammary Artery Implantation in the Treatment of Human Coronary Artery Insufficiency Including Additional Experimental Studies. J. Thorac. Surg., **29**, 1, 1955.
43) Vineberg, A.: Technical Factors Which Favor Mammary Coronary Anastomosis. J. Thorac. Surg., **30**, 411, 1955.

41) Wedel, J. V. et al.: Revascularization of the Heart by Pedicled Skin Flap. Surgery, **37**,32,1955.
45) Woodhall, B. and Golden, J.: Fibroblastic Stimulation of Rabbit Artery Adventitia by Marine Varnisch, "Krylon" and Latex. Arch. Surg., **66**, 587, 1953.
46) Yeager, G. H. and Cowley, R. A.: Studies on the Use of Polythene as a Fibrous Tissue Stimulant. Ann. Surg., **128**, 509, 1948.

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冠不全の作成に関する実験的研究

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(1) 冠不全の外科的療法,その他本症に関する諸研究を行うためには,動物に於て実験的に冠不全を作成することが緊要と考えられて来たが,我々は今回,生体内で旺盛な肉芽形成作用を有するDicetyl phosphateを利用することにより(Fig. 1, 2),漸進的冠狭窄犬の作成に成功した.

(2) 作成方法は,Dicetyl phosphateを含有しているスポンゼルを鋼線とアルミ板から成る枠の中で犬の左冠動脈前下行枝(又は廻旋枝)起始部に沿つて装着するのであつて(Fig. 3),術後肉芽が漸次増大するにつれて,その部の冠動脈が徐々に漸進的に圧迫を蒙る如くにしたのである(Fig. 4).

(3) 32頭の実験犬中,術後12日日迄の早期に屠殺した5頭では,Schlesingerによる血管造影及び病理組織学的検索によつて,未だ冠動脈狭窄が認められなかつたが,術後16日乃至56日に屠殺した27頭では,その22頭に於て明瞭な冠動脈狭窄が証明された(Fig. 11, 13).即ち,冠動脈の断面積がもとの25%以下に著明に狭窄されたものが12頭,50%以下になつたものが7頭狭窄は見られたが50%に達しなかつたものが3頭であつて,残りの5頭では狭窄は殆んど形成されていなかつた(Table I).

(4) 実験犬19頭について心電図学的追求を行つたがその16頭に於て術後2～3週間目頃から心室性期外収縮,ST上昇又は下降,T逆転等の心筋乏血を示す諸変化が認められ(Fig. 6, 7),これらの犬は何れも前述の比較的高度の狭窄を示した例に属していた.

更に7頭について,10%低酸素負荷試験を試みたが高度の狭窄を示した4頭に於ては,著明な二連脈やTv_4の逆転が発生したのに反し(Fig 8, 9),狭窄作成が失敗に終つた3頭では本試験によつて何らの変化も認められなかつた.

(5) 狭窄冠動脈配下の心筋の病理組織学的検索では,心筋梗塞が認められたものは3頭で,斑紋状結合織増殖が見られたのは6頭であつた(Fig. 18). 而して空胞変性,核萎縮,毛細管の拡張等は大多数例認められた(Fig. 19). これらの変化は何れも心筋の乏血を示す所見と考えられるものである.

(6) 本法による冠動脈狭窄の発生機序を考察するに鋼線とアルミ板から成る枠の中で肉芽が漸次増大するにつれて,冠動脈が鋼線によつてアルミ板の方向へ吊り上げられてくびれるので先ずこの機械的作用によつて狭窄が発生するが(Fig. 10, 11, 12),次いで血管周囲に結合織の増殖,この結合織の一部の血管壁内への侵入(Fig. 13 a),一方内膜の肥厚,血栓の形成等の器質的変化が二次的に加わることによつて(Fig. 14, 15, 16, 17),この狭窄が維持され,又は促進されて行くものと考えられる.

(7) 以上の如く,我々が作成した漸進的冠動脈狭窄犬は人間に於ける冠不全の臨床像をよく現出しておりしかもこの作成技術は容易で成功率は凡そ80%を示し1ヵ月内外の短期間で冠狭窄が略完成するので,今後冠不全に関する実験的研究を進める上に極めて有利な方法と信ずるものである.